EXHIBIT D

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Severe Hypercholesterolemia, Impaired Fat Tolerance and Advanced Atherosclerosis in Mice Lacking Both LDL Receptor-Related Protein 5 (LRP5) and Apolipoprotein E *

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Running title: Mice lacking both LRP5 and apoE

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SUMMARY

LDL receptor-related protein 5 (LRP5) plays multiple roles including embryonic development and bone accrual development. Recently we demonstrated that LRP5 is also required for normal cholesterol metabolism and glucose-induced insulin secretion (Fujino *et al.*, Proc. Natl. Acad. Sci. USA vol. 100, 229-234, 2003). To further define the role of LRP5 in the lipoprotein metabolism, we compared plasma lipoproteins in mice lacking LRP5, apolipoprotein E (apoE), or both (apoE;LRP5 double knockout). On a normal chow diet, the apoE;LRP5 double knockout mice (older than four months of age) had approximately 60% higher plasma cholesterol levels compared with the age-matched apoE knockout mice. In contrast, LRP5 deficiency alone had no significant effects on the plasma cholesterol levels. HPLC analysis of plasma lipoproteins revealed that cholesterol levels in the VLDL and LDL fractions were markedly increased in the apoE;LRP5 double knockout mice. There were no apparent differences in the pattern of apoproteins between the apoE knockout mice and the apoE;LRP5 double knockout mice. The plasma clearance of intragastrically loaded triglyceride was markedly impaired by LRP5 deficiency. The atherosclerotic lesions of the apoE;LRP5 double knockout mice aged six months were approximately three-fold greater than those in the age-matched apoE-knockout mice. Furthermore, histological examination revealed highly advanced arthrosclerosis, with remarkable accumulation of foam cells and destruction of the internal elastic lamina in the apoE;LRP5 double knockout mice. These data suggest that LRP5

mediates both apoE-dependent and apoE-independent catabolism of plasma lipoproteins.

INTRODUCTION

Genetic defects in the catabolism of plasma lipoproteins are important causes of hypercholesterolemia and atherosclerosis in humans. The prototypic diseases are familial hypercholesterolemia, caused by a defect in the LDL receptor (LDLR) [1] (1), and familial type III hyperlipoproteinemia, caused by a defect in one of the ligands for LDLR, apolipoprotein E (apoE) (2).

ApoE is hypothesized to mediate lipoprotein clearance by binding two receptors: (*i*) LDLR and (*ii*) a hepatic chylomicron remnant receptor. ApoE deficient mice (3-5) and LDLR deficient mice (6) exhibit hypercholesterolemia, but the severity and manifestations differ markedly. On a normal laboratory chow diet, the apoE knockout mice have much more profound hypercholesterolemia and develop spontaneous atherosclerosis (4).

LDL receptor-related protein 5 (LRP5) is a member of the LDL receptor family that are characterized by the presence of cysteine-rich complement type ligand binding domains. LRP5 binds apoE-containing lipoproteins *in vitro,* and is widely expressed in many tissues including hepatocytes, adrenal gland and pancreas (7).

LRP5 and its homologue, LRP6, are postulated to play as co-receptors for Wnt receptors, Frizzled (8-13). The Wnt signaling pathway plays an essential role in embryonic development (14,15) and oncogenesis (16) through various signaling molecules including Frizzled receptors (17), LRP5 and LRP6 (8-13) and Dickkopf proteins (11,12,18). The Wnt signaling is also involved in adipogenesis by

negatively regulating adipogenic transcription factors (19). Recent studies have revealed that loss of function mutations in the LRP5 gene cause the autosomal recessive disorder osteoporosis-pseudoglioma syndrome (OPPG) (20). Consisting with human OPPG, LRP5 knockout mice generated by Kato *et al.* exhibit a severe low bone mass phenotype (21).

Recently, we demonstrated that LRP5 deficient mice develop high plasma cholesterol levels after feeding a high-fat diet, due to decreased hepatic clearance of chylomicron remnants (22). The hepatic clearance of apoE-rich chylomicron remnants was also markedly decreased in the LRP5 knockout mice. These data suggested that LRP5 plays a role in the hepatic clearance of chylomicron remnants. In addition, we showed that the LRP5 deficient mice fed a normal diet showed marked impaired glucose tolerance. The LRP5 deficient islets had a marked reduction in the levels of intracellular ATP and Ca^{2+} in response to glucose, thereby glucose-induced insulin secretion was decreased (22). Together with the roles of LRP5 in the bone accrual development (20,23,24) as well as in the Wnt signaling pathways (8-11,13), our data indicated that LRP5 is a multifunctional receptor physiologically linked to common human disorders, including hypercholesterolemia and impaired glucose tolerance.

To further define the role of LRP5 in lipoprotein metabolism, we produced double-knockout mice that are deficient in apoE as well as in LRP5 (apoE;LRP5 double knockout mice). In the current paper, we describe that superimposition of an LRP5 deficiency onto apoE deficiency increased plasma cholesterol beyond the level observed with apoE deficiency alone. We also show that fat tolerance was markedly

impaired in the LRP5 knockout mice as well as in the apoE;LRP5 double knockout mice. Consistent with extreme hypercholesterolemia, severe atherosclerosis developed in the apoE;LRP5 double knockout mice. These results provide further evidence for the role of LRP5 in the catabolism of plasma lipoproteins.

EXPEIMENTAL PROCEDURES

Materials---For the lipoprotein analysis, blood was collected from the retroorbital plexus after a four hours fasting. Plasma total cholesterol levels were determined in individual mice at each time point by enzymatic assay kits (Wako Pure Chemical Co, Japan).

For the detection of cholesterol and triglycerides with the HPLC method (see below), we obtained enzymatic reagents from Kyowa Medex Co. (Tokyo, Japan). The reagent system for cholesterol detection consists of reagent 1 (R1-C) and reagent 2 (R2-C): R1-C, 20 mM MOPS, pH 7.0, 1.1 mM *N*-ethyl-*N*-(3-methylphenyl)- *N'*-succinylethylendiamine (EMSE), 10 U/ml peroxidase, detergents, and stabilizer; R2-C, 20 mM MOPS, pH 7.0, 1.5 mM 4-aminoantipyrine, 0.68 mM $CaCl_2$, 0.3 U/ml cholesterol esterase, 2 U/ml cholesterol oxidase, 10 U/ml peroxidase, detergents, and stabilizer. The triglyceride reagent system includes reagent 1 (R1-TG) and reagent 2 (R2-TG): R1-TG, 50 mM PIPES, pH 6.2, 1.1 mM EMSE, 2 mM $MgSO_4$, 4.9 mM ATP, 3 U/ml glycerol kinase, 1.5 U/ml glycerol-3-phosphate oxidase 5 U/ml peroxidase, detergents and stabilizer; R2-TG, 50 mM PIPES, pH 6.2, 1.5 mM 4-aminoantipyrine, 2 mM $MgSO_4$, 3U/ml lipoprotein lipase, 5U/ml peroxidase, detergents, and stabilizer. Equal amounts of R1 and

R2 were mixed before use. After mixing, the cholesterol reagent was used within four weeks and the triglyceride reagent within two weeks.

Lipoprotein analysis by a dual detection HPLC system---Plasma lipoproteins were analyzed by an improved high performance liquid chromatography (HPLC) analysis according to the procedure as described by Usui *et al.* (25). The HPLC system consisted of an AS-8020 auto-injector, CCPS and CCPM-II pumps, and two UV-8020 detectors (Tosoh, Japan) (26). An SC-8020 system controller (Tosoh) was used for instrument regulation and data collection. Lipoproteins were fractionated on two tandem connected TSKgel LipopropakXL columns (300 x 7.8 mm, Tosoh) with 50 mM Tris-acetate, pH 8.0 containing 0.3 M sodium acetate, 0.05% sodium azide, and 0.005% Brij-35 at a flow rate of 0.7 ml/min. The TSK column medium is composed of porous polymermatrices with a nominal bead size of 10 μm and a pore size of 100 nm, which is expected to exclude most of chylomicron (CM) to the void volume. Two TSK columns were connected in tandem and used to obtain higher resolution within a relatively short analytical time. The running buffer was filtered through a 0.22 μm filter (Millipore Co., Bedford, MA) before use and continuously degassed with a SD-8022 on-line degasser (Tosoh) during analysis. The column effluent was split equally into two lines by a Micro-Splitter P-460 (Upchurch Scientific Inc., Oak Harbor, WA), one mixing with cholesterol reagent and the other with triglyceride reagent, in order to achieve simultaneous profiles from a single injection. The two enzymatic reagents were each pumped at a flow rate of 0.35 ml/min for the TSK column. Both enzymatic reactions proceeded at 37 °C in a reactor

coil (Teflon, 15 m x 0.4 mm id). Ten µl samples diluted with saline were injected by an AS-8020 auto-injector with a pre-suction volume of 25 µl at intervals of 24 min. The enzymatic determination of cholesterol and triglycerides involved the detection of hydrogen peroxide produced by cholesterol oxidase and glycerol-3-phosphate oxidase, respectively. Total cholesterol and triglyceride concentrations (in mg/dl) were calculated by comparison with total area under the chromatographic curves of a calibration material of known concentration.

SDS polyacrylamide gel electrophoresis---Total lipoprotein fractions ($d < 1.215$ g/ml) from pooled plasma of the mice were isolated by ultracentrifugation, and the delipidated apolipoproteins were boiled for 3 min in SDS sample buffer containing 2-mercaptoethanol, and subjected to electrophoresis on an SDS/5-15% polyacrylamide gel. Proteins were stained with Coomassie blue.

Fat Tolerance Test---Six-month-old male mice were fasted for 16 h and olive oil (1 ml/30 g body weight, Wako Pure chemicals Co., Osaka, Japan) was administered intragastrically as a bolus. Approximately 50 µl of blood was taken from the tail vein at the indicated times for the measurement of triglyceride levels and HPLC analysis.

Mice---LRP5 "knockout" mice (originally C57BL/6J-CBA hybrids, ref. 22), LRP5-/-, have been continually mated with C57BL/6J: N6 and N7 generation descendents from this cross into the C57BL/6J background were used. ApoE-/- mice (3) backcrossed ten times on the C57BL/6J background were obtained from the Jackson Laboratory (Bar Harbor, ME). To obtain knockout mice that are homozygous

for disruption of both the LRP5 and apoE loci, male apoE-/- mice were mated to female LRP5-/- mice. The resulting apoE+/-; LRP5+/- mice were identified by PCR analysis and bred each other to produce apoE-/-; LRP5-/- mice. Experiments were performed with those mice or those with the same genotype from the next generation by breeding apoE-/-; LRP5-/- each other. Mice were maintained on 12-h dark/12-h light cycles and had free access to a normal laboratory chow diet (4.5% fat, 0% cholesterol, CE-2, CLEA, Tokyo, Japan) and water.

Measurement of atherosclerotic lesions---Mice were euthanized, and thoracic and abdominal aorta were used for *en face* staining with oil Red O to visualize neutral lipid (cholesteryl ester and triglycerides) accumulation. In brief, the aorta was removed, cleaned and cut open with the luminal surface facing up, then immersion-fixed in 10% formalin in 10 mM PBS. After rinsing with PBS, the aorta was thoroughly cleaned of adventitial fat using micro forceps and spring iris scissors under a stereoscopic microscope. The inner aortic surface was stained with oil Red O for 25 min at room temperature. After rinsing with 60% isopropanol and distilled water, the oil Red O-stained area was quantified by NIH-Image 1.62 f software analysis of the digitized microscopic images. Results are expressed as percentage of lipid accumulating lesion area of the total aortic area analyzed.

For light microscopy, the aortic tissue samples were fixed with 10% formalin in 10 mM phosphate buffer (pH7.2) and embedded in paraffin. Sections 2-3-μm thick were taken longitudinally through the aortic lumen and stained with hematoxylin and eosin (HE) or elastica-Masson (EM). For oil Red O

staining, aortic tissue samples were frozen in OCT compound (Miles Inc., Elkhart, IN). Cryostat tissue sections were cut to a thickness of 5 μm and stained with oil Red O. Nuclei were counterstained with hematoxylin.

RESULTS

Plasma cholesterol and lipoprotein profile---Fig. 1 compares the levels of total cholesterol of mice of four different genotypes at the indicated ages. Mice were fed a normal laboratory chow diet containing 4.5% (w/w) fat and 0% cholesterol. Although there was no significant differences in the total plasma cholesterol levels between the apoE knockout mice (apoE-/-;LRP5+/+) and the apoE;LRP5 double knockout mice (apoE-/-;LRP5-/-) at two months of age, the cholesterol levels of the double knockout mice older than four months were greatly increased (approximately by 60%) beyond the levels observed with apoE deficiency alone. In contrast, LRP5 deficiency alone had no significant effects on the plasma cholesterol levels.

High resolution HPLC analysis (25) of plasma lipoprotein of four-month-old mice revealed that cholesterol levels in the VLDL and LDL fractions were markedly increased in the apoE;LRP5 double knockout mice compared with the apoE knockout mice (Fig. 1B and Table I): the cholesterol levels in the VLDL and LDL fractions in the apoE knockout were 180 ± 35 and 145 ± 7 mg/dl, respectively; and those in the apoE;LRP5 double knockout mice were 244 ± 24 and 171 ± 21 mg/dl, respectably (TABLE

I). There were no significant differences in the levels of chylomicron (CM)- and HDL-cholesterol between the apoE knockout mice and the apoE;LRP5 double knockout mice, although HLD-cholesterol levels in these mice were approximately 50% of those in the LRP5 knockout mice and normal controls. Despite the severe hypercholesterolemia in the apoE knockout and apoE;LRP5 double knockout mice, there were no significant differences in the total triglyceride levels among mice with the four different genotypes (data not shown).

Fig. 2 shows the SDS polyacrylamide gel electrophoresis of apoproteins in pooled lipoprotein fraction from mice of four different genotypes. Consistent with the previous work by Ishibashi *et al.* (27), the amounts of apoB48 were markedly increased in the apoE knockout mice as well as in the apoE;LRP5 double knockout mice. Despite the severe hypercholesterolemia in the apoE;LRP5 double-knockout mice, there were no apparent differences in the pattern of apoproteins between the apoE- and apoE;LRP5 double-knockout mice.

Fat tolerance test---In the pervious study, we have shown that LRP5 plays a role in the hepatic uptake of dietary cholesterol. The LRP5 knockout mice displayed dietary derived hypercholesterolemia due to decreased plasma clearance of chylomicron remnants (22). To further define the role of LRP5, fat tolerance test was carried out using mice of four different genotypes. Mice were fasted for 16 h and olive oil (1 ml/30 g body weight) was administered intragastrically. As shown in Fig. 3, plasma levels of total triglyceride increased and peaked at about 2 h, and then declined toward baseline 6 h after loading in both

apoE-knockout mice and normal controls. In contrast, the increased levels of plasma triglyceride were sustained for several h after loading in both LRP5 knockout and apoE;LRP5 double knockout mice, indicating that the plasma clearance of intragastrically loaded triglyceride was markedly impaired by LRP5 deficiency. HPLC analysis of plasma lipoproteins revealed that the majority of particles at 6 h after fat loading were in the VLDL fraction.

In addition, we noticed that 16 h fasting increased the levels of VLDL-triglyceride in the apoE knockout, LRP5 knockout and apoE;LRP5 double knockout mice. This result may indicate that both apoE and LRP5 mediate the plasma clearance of VLDL-triglyceride induced by fasting.

Atherosclerosis---Aortic atherosclerotic lesions of the apoE knockout and apoE;LRP5 double knockout mice were first analyzed by *en face* lipid staining (Fig.4A). At four months of age, the area of the thoracic and abdominal aortas stained by oil Red O of the apoE;LRP5 double knockout mice was approximately the same as that in the apoE knockout mice. In contrast, at six months of age, the lesions in the apoE;LRP5 double knockout mice were approximately three-fold larger than those in the apoE knockout mice (Fig.4B).

In histopathology under light microscopic examination, the lesions in the apoE knockout mice at six months of age were relatively modest, showing slightly atheromatous lesions with a fatty streak-like structure, which were localized on the surface of aortic intima, but were not associated with the destruction of internal elastic lamina or the medial muscle layer (Fig. 4C). In contrast, the apoE;LRP5

double-knockout mice developed multiple atheromatous lesions manifesting a hump structure, which were associated with cholesterin deposits, fibrosis and elastosis (Fig. 4D). Some of them showed the destruction of internal elastic lamina and the degenerative change of medial muscle layers of the aorta (Fig. 4E). In these lesions severe deposition of neutral lipid was observed (Fig, 4F).

DISCUSSION

In the present study, we show extreme hypercholesterolemia in mice lacking both apoE and LRP5. It has been well established that both LDLR and apoE are critical in the plasma clearance of cholesterol-carrying lipoproteins, including LDL and apoE-containing IDL and chylomicron remnants (1,2). In contrast to the mice lacking apoE (3-5) or LDLR (6), the lack of LRP5 alone did not increase the plasma levels of cholesterol on a normal diet, while high-fat feeding results in hypercholesterolemia in the LRP5 knockout mice (22). Ishibashi *et al.* showed that the plasma cholesterol levels in the double-knockout mice lacking both apoE and LDLR, were not significantly different from the levels in the apoE knockout mice (27). The severe hypercholesterolemia developed in the double knockout mice lacking both apoE and LRP5 suggests the presence of an alternative pathway for cholesterol catabolism mediated by LRP5, which appears to be independent of the LDLR pathway.

Consistent with the previous work (22), the LRP5 knockout mice and the apoE;LRP5 double knockout mice displayed markedly impaired fat tolerance. In contrast, the plasma clearance of intragastrically

loaded triglyceride was not significantly impaired in the apoE-knockout mice. These observations suggest that LRP5 modulates the plasma clearance of dietary derived triglycerides in the absence of apoE by stimulating the hydrolysis of triglycerides. In this context, it is important to refer that LRP5 and LRP6 can bind Dickkopf (Dkk), an antagonist of Wnt proteins (12, 24). Dkk is involved in *Xenopus* head formation and the impaired action of Dkk at LRP5 increases bone density in humans (24). The Dkk sequence consists of two cysteine-rich domains. The C-terminal domain has the typical cysteine pattern of colipase, which is required by pancreatic lipase for the efficient lipid hydrolysis (reviewed in ref. 28). The C-terminal domain of colipase binds to the C-terminal noncatalytic domain of pancreatic lipase, which is though to stabilize an active conformation of the lipase, and is also conserved among various lipases including, hepatic and lipoprotein lipases. Detailed sequence analysis and molecular modeling of the Dkk sequence onto the colipase structure suggest that Dkk and colipase have the same disulfide pattern and very similar three-dimensional structures (28). This structural analogy implies a common function (lipid interaction), and raises the possibility that Dkk bound to LRP5 stimulates lipid hydrolysis by interacting hepatic lipase and/or lipoprotein lipase. Furthermore, the impaired fat tolerance caused by the deficiency of LRP5 may lead to severe hypercholesterolemia in the absence of apoE.

Another explanation for the impaired lipoprotein metabolism in the apoE;LRP5 double knockout mice is that LRP5 may recognize other lipoproteins, in addition to apoE-containing lipoproteins. The candidate apoproteins that may be recognized by LRP5 remains unidentified, since the LRP5 deficiency

did not significantly alter the pattern of apoproteins in the plasma lipoproteins of the apoE knockout mice or that of normal mice.

In addition to the role of LRP5 in embryonic development and bone development, our current data provide further evidence that LRP5 also plays a role in the metabolism of plasma lipoproteins. Furthermore, consistent with the marked elevation of plasma cholesterol, severe arthrosclerosis developed in the apoE;LRP5 double-knockout mice. The remarkable destruction of the internal elastic lamina seen in the lesion of the double-knockout mice is characteristic of highly advanced atherosclerosis. The apoE;LRP5 double-knockout mice manifesting extreme hypercholesterolemia and highly advanced arthrosclerosis will provide a useful animal model for the research and development of therapeutic agents against hypercholesterolemia and atherosclerosis.

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FOOTNOTES

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[1] The abbreviations used are: apoE, apolipoprotein E; BSA, bovine serum albumin; CM, chylomicron; Dkk, Dickkopf; HDL, high density lipoprotein; HPLC, high performance liquid chromatography ; LDL, low density lipoprotein; LDLR,LDL receptor; LRP, LDL receptor-related protein; OPPG, osteoporosis-pseudoglioma syndrome; VLDL, very low density lipoprotein, PBS, phosphate-buffered saline.

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FIGURE LEGENDS

FIG. 1. **Age-dependent changes in plasma cholesterol concentrations in mice with different genotypes fed a normal diet.** ***A,*** Plasma levels of total cholesterol of mice of each genotype at the indicated age were determined enzymatically after 4 h fasting. Data are mean $\pm$ S.D. of six mice. * $P <$ 0.01; Student's t test. ***B***, HPLC analysis of plasma lipoproteins. Plasma samples from mice of each genotype at four months of age were separated by HPLC, and cholesterol (solid line) and triglyceride (dashed line) contents were determined as described under "EXPERIMETAL PROCEDURES". Representative data from six animals with the indicated genotype is shown. The CM, VLDL, LDL, and HDL fractions are labeled C, V, L, and H, respectively. Free glycerol is indicated by an arrow head. The cholesterol levels in the CM, VLDL, LDL, and HDL fractions are shown in Table I.

Fig. 2. **SDS polyacrylamide gel electrophoresis of total lipoprotein fractions**. Equal volumes (1 ml) of plasma were pooled from four mice of different genotypes fed a normal diet and total lipoprotein fractions ($d <$ 1.215 g/ml) were isolated by ultracentrifugation, and the delipidated apoproteins were subjected to electrophoresis on an SDS/5-15% polyacrylamide gradient gel. Proteins were stained with Coomassie Blue. Position of migration of apoB100, apoB48, apoA-VI, apoE, and apoA1 are denoted. Representative data from four independent experiments is shown.

Fig. 3. **Effects of intragastrical fat loading on plasma triglyceride levels in mice with different genotypes.** Six males (six-month-old) of each genotype received an intragastrically administration of olive oil (1 ml/30 g body weight). At the indicated times, 50 µl of blood was taken from the tail vein and subjected to HPLC analysis. Data are mean ± S.E. of six mice. * $P < 0.01$; Student's *t* test.

FIG. 4. **Atherosclerotic lesions in apoE- and apoE;LRP5 double-knockout mice.** *Panel A*, *En face* lipid staining of aortas. Thoracic and abdominal aorta from the indicated genotype was cut open with the luminal surface facing up, and the inner aortic surface was stained with oil Red O. Representative data of each genotype is shown. Bar = 5 mm. *Panel B*, Quantitative analysis of *en face* lipid staining. The inner aortic surface area stained with oil Red O was quantified by NIH-Image 1.62 f software analysis of the digitized microscopic images. Results are expressed as percentage of lipid accumulating lesion area of the total aortic area analyzed. Data are mean ± S.D. of six mice. * $P < 0.01$; Student's *t* test. *Panels C-F,* Representative histopathological features of the aorta. Bars: 100 µm. *C*, An apoE-knockout mouse (aged six months) shows a slightly atheromatous lesion characteristic of the accumulation of foam cells, which is not associated with the destruction of internal elastic lamina (dark brown-colored) or the degenerative change of muscle layer of the aorta (elastica-Masson staining). *D*, One of the multiple atheromatous lesions developed in an apoE;LRP5 double-knockout mouse (aged six months) manifests a hump structure associated with cholesterin deposits, fibrosis (light green-colored) and elastosis (dark brown-

colored). Destruction of the internal elastic lamina adjacent with a degenerative lesion of muscle layer of the aorta is remarkable (elastica-Masson staining). *E*, An atheromatous lesion in an apoE;LRP5 double-knockout mouse (aged six months) reveals a remarkable accumulation of foam cells, especially marked in the superficial region of atheroma, and a crystal structure of cholesterin deposits (hematoxylin and eosin staining). *F*, An atheromatous lesion in an apoE;LRP5 double knockout mouse (aged six months) reveals severe deposition of neutral lipid in the aortic wall resulting in the destruction of lamellar structure of the elastic fibers (oil red O staining).

TABLE I

Plasma cholesterol profiles in mice with different genotypes.

Plasma samples from mice of each genotype at four months of age were separated by HPLC, and cholesterol contents were determined as described under "EXPERIMENTAL PROCEDURES". Values are mean ± S.D. of six mice.

Genotype	CM	VLDL	LDL	HDL
		Cholesterol mg/dl		
ApoE+/+; LRP5+/+	0.10 ± 0.12	2.26 ± 0.28	4.59 ± 1.05	39.9 ± 2.8
ApoE+/+; LRP5-/-	0.03 ± 0.02	3.58 ± 0.40	6.11 ± 1.01	41.2 ± 1.5
ApoE;LRP5+/+	0.16 ± 0.08	180 ± 35 [a]	145 ± 7 [a]	21.7 ± 3.8 [c]
ApoE;LRP5-/-	0.12 ± 0.05	244 ± 24 [a, b]	171 ± 21 [a, b]	22.9 ± 1.6 [c]

[a] $P < 0.01$ vs. ApoE+/+; LRP5+/+ and ApoE+/+; LRP5-/-

[b] $P < 0.01$ vs. ApoE-/-;LRP5+/+

[c] $P < 0.01$ vs. ApoE+/+; LRP5+/+ and ApoE+/+; LRP5-/-

Fig.1

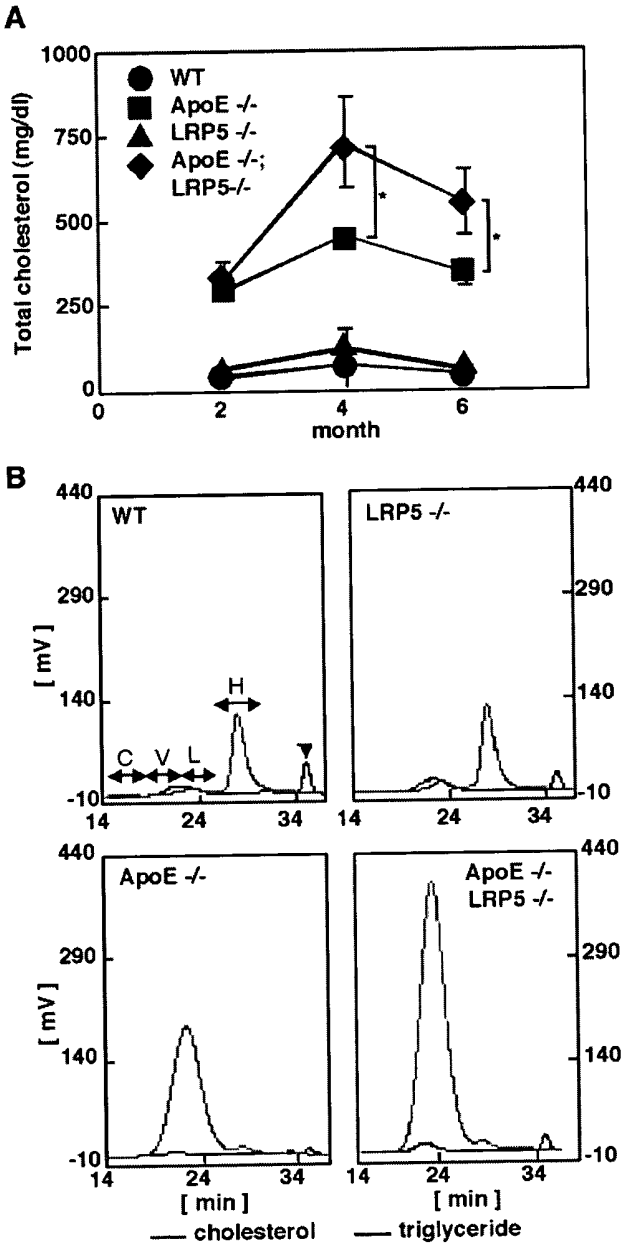

A
1000
750
500
250
0
Total cholesterol (mg/dl)
WT
ApoE -/-
LRP5 -/-
ApoE -/-;
LRP5-/-
*
*
0
2
4
6
month
B
440
290
140
-10
[mV]
WT
H
C
V
L
14
24
34
LRP5 -/-
ApoE -/-
ApoE -/-
LRP5 -/-
[min]
cholesterol
triglyceride

Fig.2

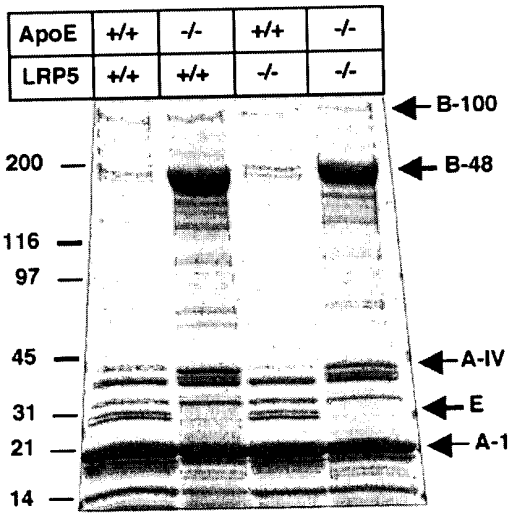


Fig.3

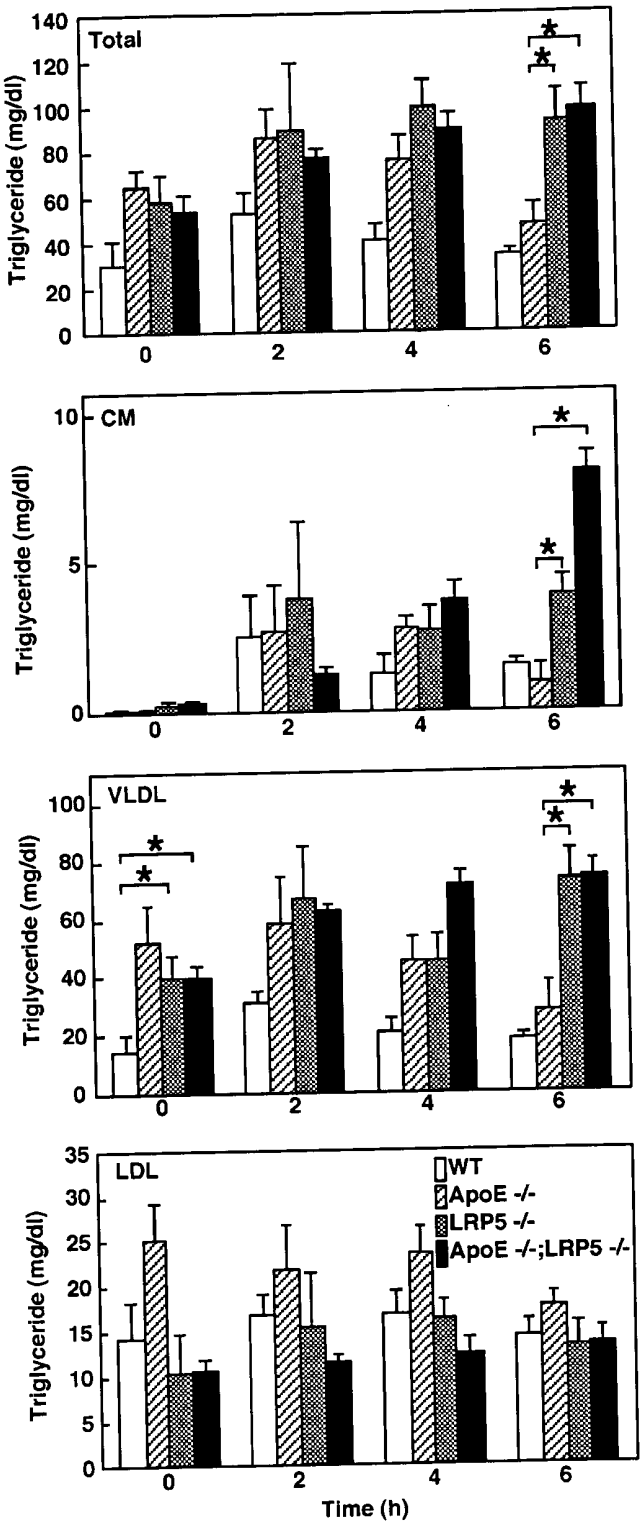
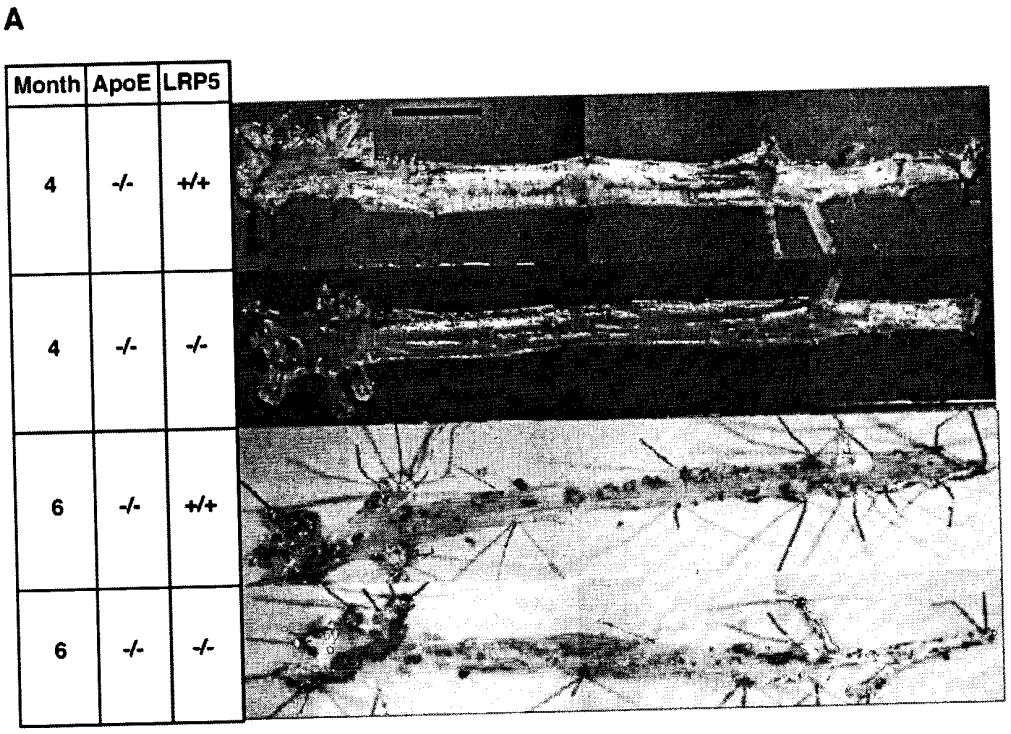
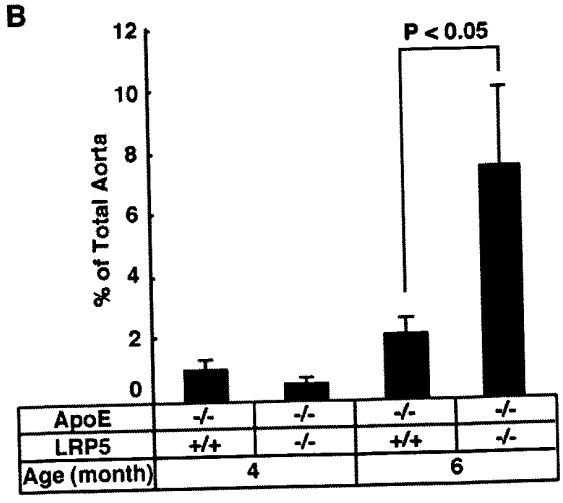

Total
CM
VLDL
LDL
Triglyceride (mg/dl)
Time (h)
WT
ApoE -/-
LRP5 -/-
ApoE -/-;LRP5 -/-

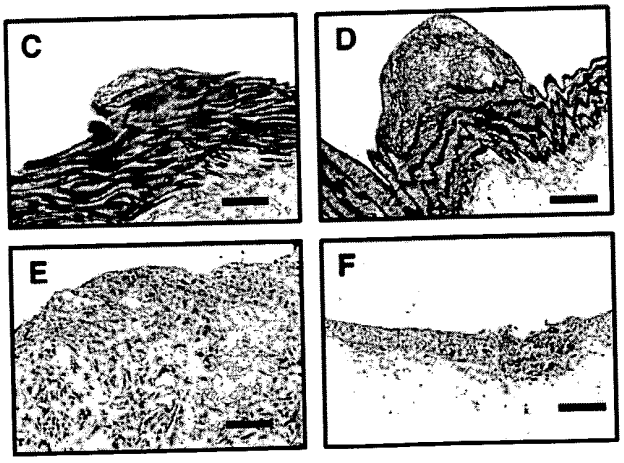
Fig.4

A


Month
ApoE
LRP5
4
-/-
+/+
4
-/-
-/-
6
-/-
+/+
6
-/-
-/-

B


P < 0.05
% of Total Aorta
12
10
8
6
4
2
0
ApoE
-/-
-/-
-/-
-/-
LRP5
+/+
-/-
+/+
-/-
Age (month)
4
6


C
D
E
F